The Ultrastructure and Evolutionary Significance of the Cerebral Ocelli of *Mytilus edulis*, the Bay Mussel

BY

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(4 Plates; 3 Text figures)

INTRODUCTION

Mytilus edulis Linnaeus, 1758, the bay mussel, has circumboreal distribution and is usually found in protected coastal waters. The life history includes planktonic trochophore, veliger, and pediveliger stages (FIELD, 1922). At a length of 240 - 245 μm, the veliger larvae develop a pair of eyes immediately posterior to the prototroch and innervated by the cerebral ganglia (BAYNE, 1964). Veligers with eyes display positive phototaxis until they approach metamorphosis, at which time they become negatively phototactic (BAYNE, *op. cit.*). The paired cerebral eyes do not atrophy during metamorphosis, as originally reported by Lovén but persist throughout life (PELSENEER, 1899, 1908).

Among bivalves, cerebral eyes are distributed throughout several superfamilies including the Mytilacea, Pteriacea, Arcacea, Anomiacea, and Ostreacea (PELSENEER, 1908). According to a phylogenetic scheme presented by STASEK (1963), all of these superfamilies represent an evolutionary offshoot that diverged early from the main line of bivalvian evolution.

Pallial eyes, located on the exposed mantle edges, are more conspicuous than cerebral eyes and have been described at an ultrastructural level in the following groups: Pectinacea (BARBER *et al.*, 1967), Cardiacea (BARBER & WRIGHT, 1969a), Tridacnacea (KAWAGUTI & MABUCHI, 1969), Arcacea (LEVI & LEVI, 1971), Pandoridae (ADAL & MORTON, 1973). Except for the Arcacea and Pectinacea, bivalves with pallial eyes represent more recently evolved groups than do bivalves with cerebral eyes (STASEK, 1963). Although the presence and position of eyes were not taken into account in the construction of Stasek's phylogenetic scheme, his analysis reflects the probability that cerebral eyes existed in bivalves prior to pallial ones. Accordingly, the cerebral eyes of bivalves may be homologous to those of the other molluscan classes, as well as to those of the other major invertebrate groups. Thus, one would expect greater similarity among the cerebral eyes of various mollusks than between cerebral and pallial eyes of bivalves.

The evolutionary significance of the fine structure of invertebrate eyes and photoreceptors has been discussed by EAKIN (1963, 1965, 1968), LAND (1968), HERMANS (1969), HERMANS & EAKIN (1974), WOLKEN (1974), ERMAK & EAKIN (1975), VANFLETEREN & COOMANS (1976), and others. Although all organelles thought to be photoreceptive are composed of membranous systems providing relatively large surface areas, EAKIN (1963, 1972) postulated that there are 2 distinctive types of photoreceptors: ciliary and rhabdomeric.

In ciliary photoreceptors, the membranous systems are formed from sensory cilia that typically lack a central pair of microtubules in the axonemes, and are referred to as 9+0. The outer membranes of these cilia give rise to the tubules, lamellae, sacs, discs, and other structures that form the photoreceptoral organelles (EAKIN, 1972). In rhabdomeric photoreceptors the organelles consist of microvilli that develop from membranous projections and invaginations of the plasmalemmas of the photoreceptor cells, not of the plasmalemmas of cilia.

EAKIN (1968) further postulated that ciliary photoreceptors are predominantly characteristic of the deuterostomous line of evolution, whereas the protostomous phyla are largely characterized by rhabdomeric photoreceptors. However, a number of inconsistencies have been demonstrated in that not all photoreceptors in protostomes are rhabdomeric and not all photoreceptors in deuterostomes are ciliary.

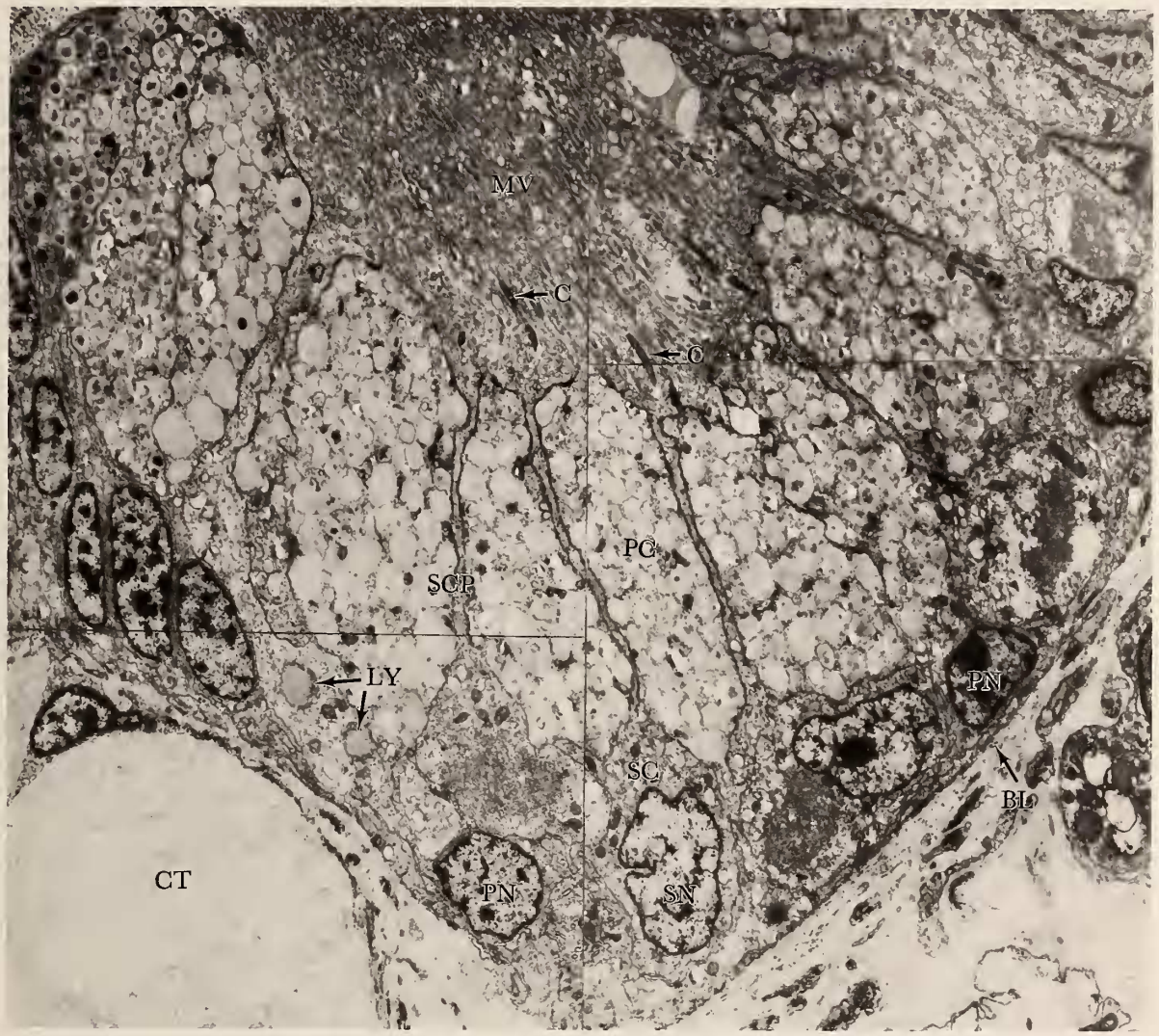

Figure 3

Low magnification electron micrograph of a transverse section through an ocellus showing the shape and arrangement of the sensory cells (SC), pigment cells (PC), and the rhabdomeric microvilli (MV). BL - basal lamina; CT - connective tissue; SCP - sensory cell process; LY - lysosomes; PN - pigment cell nucleus; SN - sensory cell nucleus; C - cilia ×4500

In contrast to Eakin, Vanfleteren & Coomans (1976) believed the differences between ciliary and rhabdomeric photoreceptors to be more of quantitative than of qualitative order. Those authors suggested on the basis of diverse evidence that the formation of both types of photoreceptors is initially induced by cilia, with the cilia being partially to completely aborted in rhabdomeric types. Thus, Vanfleteren & Coomans concluded that the type of photoreceptor is not conservative or distinctive enough for evaluating evolutionary relationships between phyla.

The presence of the same type of photoreceptor in ocelli that would not otherwise be considered homologous suggests that photoreceptor type is a trait that could have been convergently evolved. This means that homology must first be determined at an ocellar, or organ level rather than at a photoreceptoral, or organelle level. Some authors have noted that the ultrastructural similarities among the cerebral ocelli of various sipunculans, mollusks, annelids, and onychophorans extend beyond the resemblance of their rhabdomeric photoreceptors (Hermans, 1969; Hermans & Eakin, 1974; Ermak & Eakin, 1975). These cerebral ocelli are regarded by those workers as likely to be homologous. Thus, cerebral eyes may provide a coherent line of evidence with which to evaluate interphyletic relationships.

The purpose of this study is to offer the first ultrastructural description of the cerebral ocellus of a bivalve mollusk, to appraise its evolutionary significance, and to reassess the value of photoreceptors in the study of evolution.

We wish to express our appreciation to Dr. Richard M. Eakin for his counsel during the course of this investigation.

MATERIALS AND METHODS

Specimens of *Mytilus edulis* ranging in length from 3 mm to 3 cm were collected at Mason's Marina, Bodega Bay, Sonoma County, California. The mussels were either fixed immediately or maintained at 12° C in aquaria of unfiltered seawater.

Living mussels were bisected along the sagittal plane and placed in a fixative solution of 4% glutaraldehyde, 0.15M sodium cacodylate and 0.15M sodium chloride at room temperature and pH 7.3. After 30 minutes in this solution, the eyes were excised and transferred to a fresh solution of the same fixative for 1 hour; washed for 30 minutes in a solution of 0.15M sodium cacodylate and 0.15M sodium chloride at room temperature and pH 7.3; and post-fixed for 1 hour in ice cold 2% OsO_4, 0.15M sodium cacodylate and 0.15M sodium chloride at pH 7.3. Dehydration in ethanol and propylene oxide was followed by embedding and sectioning in Epon.

For light microscopy, sections 1 μm thick were mounted on glass slides and stained with 1% toluidine blue in 1% borax.

For electron microscopy, gold and silver ultrathin sections were collected on uncoated grids and stained in uranyl acetate and lead citrate. The sections were examined with a Zeiss EM 9A having an accelerating voltage of 60 kv.

RESULTS

General Anatomy

The paired cerebral eyes of *Mytilus edulis* are located at the bases of the first ctenidial filaments of left and right inner demibranchs (Figures 1 and 2, O). The eyes in living specimens are covered with mucus from the ctenidia, and each appears as a dark reddish-brown spot when viewed with a hand lens. The diameter of the eye is 40 - 50 μm throughout the life of the mussel, with the result that the eyes of small specimens are more conspicuous than those of large ones.

Lateral to each eye is a triangular translucent zone in the anterior region of each valve (Figure 2, SW). These "shell windows" permit diffuse light to reach the eyes. In mussels less than 5 mm long, the entire shell is thin and translucent. In larger mussels, with thicker shells, only the shell windows remain translucent.

Microscopic Anatomy

Each ocellus is an "open cup" with no cornea or lens (Figure 6). The opening of the cup faces laterally into the space between the inner demibranch and outer palp (Figure 6, ID, OP). Cilia from adjacent feeding organs project into this space (Figure 6, C). A layer of fibrous connective tissue lies below the eye (Figure 6, CT). The retina, composed of sensory and pigment cells, has 3 distinct regions or layers: nuclear, pigmented, and photoreceptoral (Figure 6, a, b, c). In the nuclear region, 8 μm thick, both sensory and pigment cell nuclei are visible. The former are larger and stain lighter than the latter. In the pigmented region, 20 μm thick, the pigment cells (Figure 6, PC) are broad and lightly stained, whereas the sensory cells (Figure 6, SC) are narrow and stain more darkly. The photoreceptoral region is 15 μm thick and consists of photoreceptoral organelles that are directed toward incoming light rather than away from it. Thus, the ocellus is converse rather than inverse.

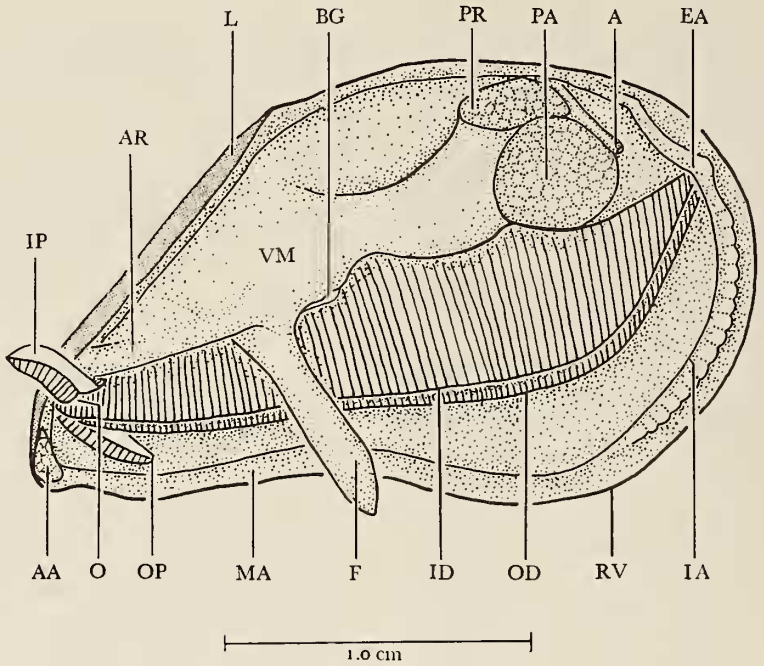

Figure 1

Side view of *Mytilus edulis* with left valve and pallial organs removed. The cerebral ocellus (O) (not visible) is located behind the inner palp (IP) at the base of the first filament of the inner demibranch (ID). AR – anterior retractor muscle; L – hinge ligament; BG – byssus gland; VM – visceral mass; PR – posterior retractor muscle; PA – posterior adductor muscle; A – anus; EA – exhalant aperture; IA – inhalant aperture; RV – right valve; OD – outer demibranch; F – foot; MA – mantle; OP – outer palp; AA – anterior adductor muscle

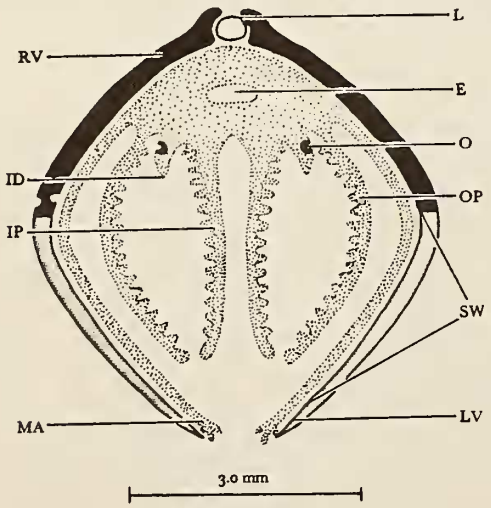

Figure 2

Transverse section through the first anterior filaments of left and right inner demibranchs (ID), showing the location of the ocelli (O) and shell windows (SW). E – esophagus; LV – left valve. See Figure 1 for additional symbols

Ultrastructure

The pigment and sensory cells interdigitate in an irregular manner (Figures 3 and 4). A basal lamina separates the retina and adjacent ciliated epithelium from the underlying connective tissue (Figure 3, BL, CT). The nuclear regions of the pigment cells average 3.0 μm in width. Distal to the nuclear regions these cells are heavily pigmented and collectively form the pigment cup. Here, each cell is about 4.0 μm wide. Pigment cell apices narrow to approximately 1.0 μm and give rise to stubby microvilli that average 0.6 μm in length and 0.13 μm in diameter. The pigment cells are all close to 23 μm long, including microvilli.

The sensory cells have bulbous nuclear regions that average 4.0 μm in width. Distal to the nuclear region, slender sensory cell processes, approximately 0.3 μm wide, occupy the spaces between the heavily pigmented areas of the pigment cells (Figure 3, SCP). Apically, sensory cells each expand to a width of about 3.0 μm and give rise to the rhabdomeres, which consist of parallel arrays of undulating microvilli. Each microvillus is about 12 μm

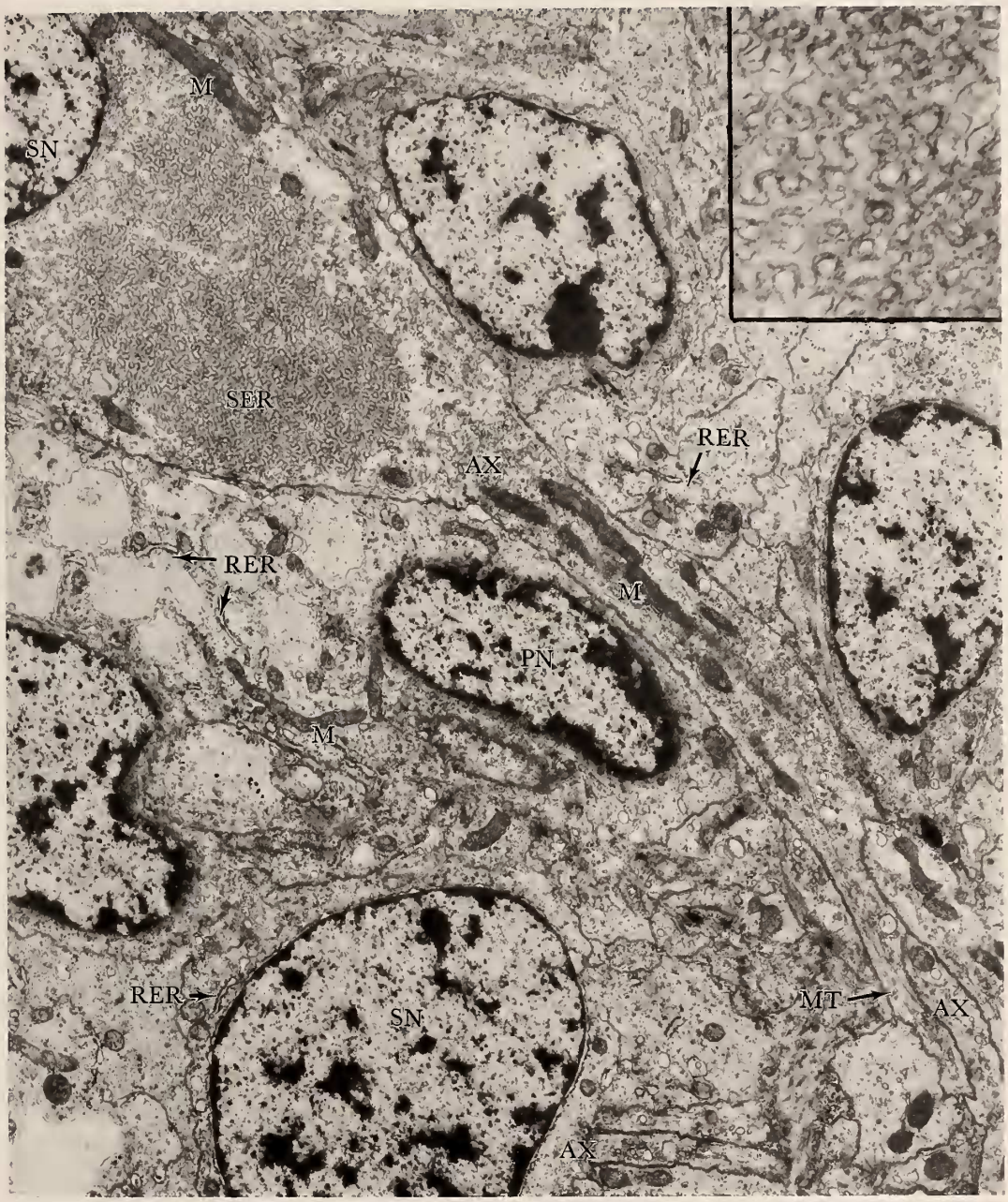

Figure 5

Nuclear region of the ocellus showing an axon (AX) in continuity with the proximal part of a sensory cell, and two other axons converging at lower right. M - mitochondria; RER - rough endoplasmic reticulum; SER - smooth endoplasmic reticulum; MT - microtubules; SN - sensory cell nucleus; PN - pigment cell nucleus. ×10 000

Inset: Enlargement of unusual smooth endoplasmic reticulum (SER) ×38 000

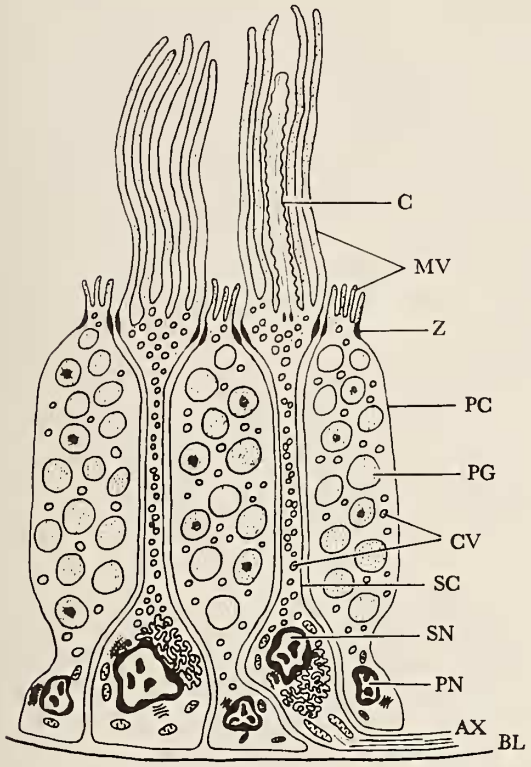

Figure 4

Part of retina illustrating the shape and arrangement of sensory cells (SC) and pigment cells (PC). C – cilium; MV – microvilli; Z – adhering zonule; PG – pigment granule; CV – cytoplasmic vesicles; SN – sensory cell nucleus; PN – pigment cell nucleus; AX – axon; BL – basal lamina

long and 0.16 μm in diameter (Figures *3* and *12*, MV). The overall length of these cells, including the rhabdomeres, averages 35 μm.

In sensory cells mitochondria are numerous only in the nuclear region (Figures *5* and *7*, M). Membrane-bounded cytoplasmic vesicles are abundant distal to the nuclei (Figure *7*, CV). Lysosome-like organelles have been occasionally observed (Figure *3*, LY). Rough endoplasmic reticulum and an unusual type of smooth endoplasmic reticulum are frequently seen (Figure *5*, RER, SER). The unusual smooth endoplasmic reticulum consists of irregular crenulations of 2 undulating membranes separated by a uniform space of 175Å (Figure *5*, inset). This organelle has only been observed close to the sensory cell nucleus. Axons originate from the proximal ends of the sensory cells (Figure *5*, AX). The axons contain numerous mitochondria, longitudinally oriented microtubules, and finely granular cytoplasm (Figure *5*, M, MT).

Sensory cell processes contain numerous membrane-bounded cytoplasmic vesicles from 0.1 to 0.3 μm in diameter, which have heterogeneously dense centers (Figure *8*, CV). Longitudinally oriented microtubules and dense granules, averaging 400Å in diameter, which are presumed to be beta particles of glycogen, were also observed (Figure *8*, MT, GG).

Pigment cells contain fewer organelles than do sensory cells, with mitochondria and rough endoplasmic reticula occupying the nuclear regions (Figure *5*). Distal to the nuclear region, pale membrane-bounded granules of shading pigment are the main constituents of these cells (Figures *7* and *8*, PG). The pigment granules range from 0.7 to 2.0 μm in diameter and their densities vary. Such granules are absent from the sensory cells. Dispersed throughout the pigment cells are membrane-bounded cytoplasmic vesicles similar to, but fewer in number than, those observed in sensory cells (Figure *8*, CV).

Sensory and pigment cell apices are characterized by prominent adhering zonules and septate junctions (Figures *9*, *10*, and *11*, Z, SJ). The septate junctions form borders between sensory cell processes and pigment cells that extend from the apices well into the pigmented region of the retina (Figures *9* and *10*, SJ). Cytoplasmic vesicles are located at the apices of both cell types (Figures *10* and *11*, CV). Some pigment cell apices contain unusually dense cytoplasm (Figure *10*, DC). A single cilium, oriented parallel to the microvilli, is frequently observed originating from a sensory cell (Figure *11*, C). Observed in cross section, such cilia clearly show the typical 9+2 arrangement of microtubules in their axonemes (Figure *13*, C). Cilia have not been observed to originate from pigment cells.

DISCUSSION

Structure

Cerebral ocelli of bivalve mollusks have been described at a light microscopical level by Pelseneer (1899, 1908), Field (1922), and others, as cited by Bayne *et al.* (1976). Pelseneer (1908) compared the cerebral eyes of 30 closely related bivalves and found their structure to be similar and their location uniform. Both Pelseneer and Field concluded that the ocelli of mussels are well developed and capable of directional sensitivity to light. Both of these authors, however, described a cuticular lens which the present electron micro-

scopical investigation demonstrates to be the array of photoreceptoral microvilli. The ocellar cavity has no cuticular covering. The ocelli of *Mytilus* are "open cups" similar to those of *Haliotis* (Tonosaki, 1967) and *Nautilus* (Barber & Wright, 1969b) in which the ocellar cavities are exposed directly to seawater.

In the absence of a lens, the ocellus of *Mytilus* is not so well-developed as Pelseneer and Field concluded. Well-developed invertebrate eyes, presumed to be capable of forming images, typically possess a lens such as that found in the pelagic polychaete *Vanadis tagensis* Dales, 1955 (Hermans & Eakin, 1974). In contrast to this, the eye of *Nautilus*, a primitive cephalopod, lacks a lens but is able to cast inverted images onto the retina by means of a pinhole aperture (Barber & Wright, 1969b; Wolken, 1971). Lacking a lens or pinhole aperture, the ocellus of *Mytilus* is not capable of forming images. Nevertheless, it is possible that the ocelli give *Mytilus* limited sensitivity to the direction of illumination, as interpreted from the regular organization of the microvillous rhabdomeres and the concavity of the retinas.

Functions of the sensory and pigment cells are directly related to their differing forms (Figures 3 and 4). The broad apices of the sensory cells give rise to the microvilli, which are presumed to be photoreceptoral organelles (Eakin, 1972). Slender sensory cell processes, passing through the pigmented region, contain microtubules and numerous cytoplasmic vesicles as supportive or transportive elements (Eakin, 1972). These processes are without pigment granules and therefore do not function as shading elements. The relatively wide nuclear regions of the sensory cells house rich accumulations of mitochondria and both smooth and rough endoplasmic reticula. The presence of these organelles indicates the high level of synthetic activity common to visual cells (Eakin, *op. cit.*). Pigment cells are narrow apically and bear microvilli that are and presumed to be of a supportive function. These cells are maximally broad in the pigmented region, forming the pigment cup whose presumed function is that of shading the rhabdomeres from light in all but one direction. It is unlikely that the pigment cup also serves as a reflecting layer, for it lacks the characteristics of a tapetum (Eakin, *op. cit.*). The reduced number of organelles in the pigment cells indicates a level of synthetic activity lower than that in sensory cells.

The interdigitation of sensory and pigment cells is not entirely regular. Two or more cells of the same type are occasionally observed together. This irregularity is also found in some gastropods: *Littorina scutulata* Gould, 1848 (Mayes & Hermans, 1973); *Haliotis discus* Reeve, 1846 (Tonosaki, 1967); *Littorina littorea* Linnaeus, 1758; *Nucella emarginata* Lamarck, 1819; *Lacuna* sp.; *Tegula funebralis* Adams, 1854 (all in Baker, 1975); *Helix aspersa* Müller, 1774 (Eakin & Brandenburger, 1967a). In the retinas of squids and octopuses, however, the interdigitation is regular; there is a single pigmented-supportive cell for every tetramerous rhabdome (Zonana, 1961; Tonosaki, 1965). A relatively uncommon feature of retinas composed of interdigitating sensory and pigment cells is the total lack of pigment granules in the sensory cells. The ocelli of certain gastropods (Eakin, 1968) and of the sea star *Asterias rubens* Linnaeus, 1758 (Vaupel-von Harnack, 1963) share this feature with *Mytilus*, as well as a marked similarity in the arrangement and relative form of the retinal cells.

Red pigment granules are common to the ocelli of *Mytilus*, of the protist *Euglena* (Wolken, 1967), and of the sea stars *Henricia leviuscula* Stimpson, 1857 (Eakin, 1968) and *Asterias rubens* Linnaeus, 1758 (Vaupel-von Harnack, 1963). When fixed in glutaraldehyde, red pigment granules tend to appear very pale (Eakin, 1972). A red, water-soluble substance, taken from the ocelli of

Explanation of Figures 6 to 9

Figure 6: Light micrograph of a transverse section through an ocellus and adjacent structures: outer palp (OP); inner demibranch (ID); and connective tissue (CT). Three distinct layers are visible within the ocellus: nuclear (a); pigmented (b); and photoreceptoral (c). C – cilia; PC – pigment cell; SC – sensory cell × 1 400

Figure 7: Nuclear region of ocellus showing a portion of a sensory cell nucleus (SN) and 2 sensory cell processes (SCP). M – mitochondria; CV – cytoplasmic vesicles; PG – pigment granule; PC – pigment cell × 11 000

Figure 8: Pigmented region of the ocellus showing a sensory cell process (SCP) between 2 pigment cells (PC). CV – cytoplasmic vesicles; MT – microtubules; GG – glycogen granules; PG – pigment granule × 14 000

Figure 9: Apices of pigment and sensory cells (PC, SC). A presumptive ciliary basal body (BB) can be seen at the apex of one sensory cell. Adhering zonules (Z) and septate junctions (SJ) are prominent in this region. MV – microvilli; C – cilia × 14 000